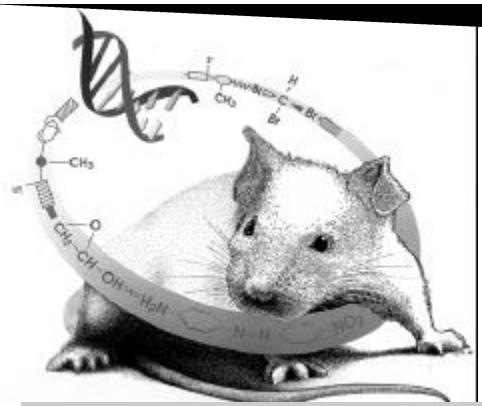

The Tg.AC Workgroup Newsletter

The Tg.AC Workgroup Newsletter is published by The Department of Toxicology and Safety Assessment, Boehringer Ingelheim Pharmaceuticals, Inc. as a means of communication for the HESI's Alternative to Carcinogenicity Testing Committee.

Letter and article submissions are welcome. Persons interested in contributing to the newsletter should contact:

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On the Inside:

ILSI Tg.AC Working Committee Update

By R.E. Stoll, BIPI and R. Tennant, NIEHS

QUESTION:
What type Tg.AC (hemizygote or homozygote) animal should I use for my upcoming ILSI study?

BACKGROUND:
Back in late 1996, we were recommending use of the hemizygote Tg.AC animal. In early 1997, other investigators such as FDA and Boehringer Ingelheim Pharmaceuticals, Inc. (BIPI) observed an inconsistent response in the hemizygote. The Tg.AC working committee recommended using the homozygote Tg.AC since a more consistent reproducible positive response was noted going "across" studies. In late 1997, we (FDA, NIEHS, and Boehringer) identified that a problem existed with the hemizygotes to the point that few animals were capable of responding to a positive control. Taconic, NIEHS, FDA, Dupont and Boehringer met in March 1998 to arrive at a solution as to how to remedy the hemizygote and homozygote non-responder issue. The consensus recommendation of all parties involved was to "take down" the colonies and "restart" both colonies at the earliest time possible. Elsewhere in this Tg.AC newsletter, Taconic Farms addresses the availability of hemizygote or homozygote Tg.AC "responder" animals. For supply of non-responder-free hemizygote animals, limited numbers will be available in August. These type animals should be capable for use in ILSI investigative compound test protocols. If one wants to wait for one of the homozygote non-responder-free animals, they will be available in mid December 1998. These animals can also be used in ILSI investigative test protocols. Both type colonies will have been corrected, restarted, and provide 100% of animals that will have been genotyped via Southern blot methodology developed by the FDA, that in two previous double-blinded test protocols yielded a 1.00 predictive value in detecting a non-responder versus a responder.

ANSWER:
Thus, the answer to the question posed is that you can use either the hemizygote or homozygote for ILSI studies. Going beyond the ILSI tests being performed: if one is using the Tg.AC animal for a bona fide regulatory submission in either an IND or NDA, you may want to interact directly with the

FDA for a discussion as to which Tg.AC animal (hemizygote or homozygote) is preferred in such a setting.

Last point, irrespective of using hemizygote or homozygote animals for ILSI tests, please continue to take a tail sample (frozen at necropsy) for potential genotyping if questions are raised post termination of the study. As well, make sure a positive control group is incorporated into the design of each study.

Tg.AC Availability from Taconic

By Donna Gulezian, Taconic Farms, Inc.

Taconic is responding to client concerns regarding future shipments of Tg.AC Responder mice. This has involved tearing down the Tg.AC homozygous and hemizygous production colonies that had been supplying the Tg.AC mice of the SV40 genotype to customers. New colonies are being started with proven Tg.AC Responder mice as breeders to produce both hemizygous and homozygous Tg.AC mice (See column on test-mating). This need to restart the colonies has, of course, affected the availability of Tg.AC mice. Taconic anticipates a limited supply of Tg.AC hemizygote Responder mice beginning in the early part of August, 1998. These mice will be the offspring of hemizygous Tg.AC Responder mice bred to wild type FVB mice. This mating will produce offspring of two possible genotypes, hemizygous and wild type. Therefore, all offspring will be tested for the Tg. AC Responder genotype prior to shipment. This need to test each mouse will result in five-week old mice being the youngest ages available for shipment because of the time required to perform Southern Blot Assays and obtain genotype results. The supply of Tg.AC hemizygote mice will continue to increase in the fall and winter of 1998. Homozygote Tg.AC Responder mice are expected to be available in limited supply in mid-December, 1998. As described in the column on test-mating a number of steps are required to set up a homozygous Responder production colony of Tg.AC mice. Animals must first be intercrossed to produce putative homozygous Responder Tg.AC mice and then test-mated to prove homozygosity. Therefore, production of homozygous Tg.AC Responders will require two generations of mating. First, hemizygotes of the Tg.AC Responder gene must be mated to each other to produce offspring of three possible genotypes (25% wild type, 50% hemizygotes and 25% homozygotes). Then the carriers (i.e. hemizygotes and homozygotes) must be test mated to determine which mice are homozygotes and which are hemizygotes. This process is underway at Taconic. The supply of Tg.AC homozygote Responder mice will continue to increase in 1999. When the process is complete, homozygous and hemizygous mice will be available for shipment as young as four weeks of age. At this point Tg.AC mice that are shipped will not need to be tested by Southern Blot since the genotype of their parents will be known (i.e. both parents will be proven homozygote Responders or, in the case of hemizygotes, one parent will be a proven homozygote and the other an FVB). Southern Blot Assays will continue to be performed on all breeders to ensure the Responder genotype. As stated earlier, Taconic will Southern Blot test prior to shipment to customers all offspring from the current Tg.AC carrier x FVB colony that produces hemizygous Tg.AC Responder mice. As soon as homozygous Responder Tg.AC mice are available, Taconic will set up a Foundation Colony of proven homozygous Responders to produce Tg.AC homozygous Responder mice for use as breeders in Production colonies. Zygosity of the initial homozygous Foundation Colony breeders will be confirmed via Southern Blots and test-matings. Additionally, Taconic intends to perform phenotypic tests on offspring from these initial breeders to confirm correlation between the

genotype and phenotype. Offspring from the Foundation Colony will be used as breeders in both the homozygous and hemizygous Production Colonies. In the case of the homozygous Production Colony, offspring from the Foundation Colony will be transferred to the Isolated Barrier Unit colony and genotyped when they and placed in their breeding cage. This genotyping should confirm the carrier status of breeders for the Responder gene thus verifying that they originated from the homozygous Foundation colony. Finally, Taconic will set up a colony of proven homozygous Responders bred with wild type inbred FVB mice to produce hemizygous Responder mice. This colony will become the source for shipments of hemizygous Responder mice. Taconic will Southern Blot assay both the homozygote and the FVB parental breeders in this colony to confirm their respective carrier and wild type status.

Contact: D. Gulezian (203)245-3970

"What is a Test-Mate?"

By Donna Gulezian, Taconic Farms, Inc.

The Tg.AC Responder genotype is detected by a Southern Blot Assay that was discovered in the laboratory of Dr. Frank Sistare of the FDA as the result of an investigation to detect possible differences in the Tg. AC transgene in Responder and Non-Responder Tg. AC mice. This assay uses a probe that is based on the sequence in the zeta-globin promoter region of the Tg. AC transgene. The original Southern Blot Assay used a probe based on the SV40 region of the transgene. The zeta-globin based Southern Blot Assay can reliably identify carriers of the Tg.AC Responder genotype. That said, it is important to note that the zeta-globin promoter based assay, as is the case with other Southern Blot Assays used to determine the presence of a transgene in a microinjected transgenic model, cannot distinguish between a homozygote and hemizygote Tg.AC Responder. The assay can only determine that a mouse carries the Responder transgene. It cannot determine gene dose (whether it is present in one or both alleles) and it cannot determine transgene copy number. How then is it possible to differentiate homozygote and hemizygote Tg.AC mice? This can be accomplished by 'test-mating'. A test-mate is performed by breeding a carrier Tg.AC Responder, presumed to be a homozygote Tg.AC Responder mouse based on the known carrier status of its parents, and a wild type FVB mouse. The FVB mouse does not carry the transgene. If the resulting pups from this mating produce pups that are carriers of the Tg.AC Responder gene then the 'putative homozygote' status of the parental carrier of the Responder Tg.AC is confirmed or "proven". This homozygote Responder Tg.AC mouse can then be set-up with another proven homozygote Responder mouse to produce known homozygous Responder offspring. Alternatively, the test-mate proven homozygote Responder mouse can be bred to a wild type FVB to produce litters in which all the pups will be hemizygote Responders. There are a number of conditions that must be met before the status of putative homozygote can be changed to proven homozygote as a result of successful test-mating. To determine that a Tg.AC mouse is a homozygote Responder a minimum of two complete litters must be tail-clipped and DNA assayed from the test-mate. These two litters must have a minimum of ten pups between them, and the genotype of all pups must be confirmed as Tg.AC Responder carriers by the Southern Blot analysis. Therefore, all of these criteria must be met before determining that a test-mate proved that a Tg.AC Responder carrier is a homozygote carrier.

A minimum of two litters must be tested.
There must be a minimum of ten pups between them.
All the pups from these two litters must be tested.
All test results must be returned as positive for Tg.AC Responder carrier status.

If all of these conditions are met, the test-mate successfully indicates that the Tg.AC Responder parent was indeed a Tg.AC homozygote Responder mouse and can be used accordingly in future breeding to produce the desired Tg.AC Responder genotype.

List of Articles to be Released in a Future Toxicologic Pathology Journal

- The National Toxicology Program Evaluation of Genetically Altered Mice as Predictive Models for Identifying Carcinogens. W.C. Eastin, J.K. Haseman, J.F. Mahler, and J.R. Bucher. NIEHS.

- ILSI's Role in the Evaluation of Alternative Methodologies for the Assessment of Carcinogenic Risk. D. Robinson. International Life Sciences Institute.

- Tripropylene Glycol Diacrylate, but not Ethyl Acrylate, Induces Skin Tumors in a Twenty Week Short Term Tumorigenesis Study in Tg.AC (v-Ha-ras) Mice. L.A. Nylander-French and J.E. French. Dept. Environmental Sciences and Engineering, University of North Carolina and NIEHS.

- Carcinogenic Responses of Transgenic Heterozygous p53 Mice to Inhaled 239PuO2 or Metallic Beryllium. G.L. Finch, T.H. March, F.F. Hahn, E.B. Barr, S.A. Belinsky, M.D. Hoover, J.F. Lechner, K.J. Nikula, and C.H. Hobbs. Lovelace Respiratory Research Institute.

- Phenobarbital Does Not Promote Hepatic Tumorigenesis in a 26-Week Bioassay in p53 Heterozygous Mice. J. E. Sagartz, S.W. Curtiss, R.T. Bunch, J.C. Davila, D.L. Morris, and C.A. Alden. Searle.

- Spontaneous and Chemically-Induced Proliferative Lesions in Tg.AC Transgenic and p53-Deficient Mice. J.F. Mahler, N.D. Flagler, D.E. Malarkey, P.C. Mann, J.K. Haseman, and W.E. Eastin. NIEHS, North Carolina State University College of Veterinary Medicine, and Experimental Pathology Laboratories, Inc.

- Morphological Characteristics of Spindle Cell Tumors Induced in Transgenic Tg.AC Mouse Skin. S. Asano, C. S. Trempus, J.W. Spalding, R.W. Tennant, and M. StJ. Battalora. NIEHS.

- Pathological Featurers of Spontaneous and Induced Tumors in the Transgenic Mice Carrying a Human Prototype c-Ha-ras Gene Used for 6-Month Carcinogenicity Studies. K. Mitsumori, H. Koizumi, T. Nomura, and S Yamamoto. National Institute of Health Sciences, Central Institute for Experimental Animals, Department of Pharmacology, Keio University.

- An Evaluation of the Hemizygous Transgenic Tg.AC Mouse for Carcinogenicity Testing of Pharmaceuticals I. Evidence for a Confounding Nonresponder Phenotype. J.L. Weaver, J.F. Contrera, B.A. Rosenzweig, K.L. Thompson, P.J. Faustino, J.M. Strong, C.D. Ellison, L.W. Anderson, H.R. Prasanna, P.E. Long-Bradley, K.K. Lin, J. Zhang, and F.D. Sistare. CDER, FDA.

- An Evaluation of the Hemizygous Transgenic Tg.AC Mouse for Carcinogenicity Testing of Pharmaceuticals II. Genotyping that Predicts Tumorigenic Responsiveness. K.L. Thompson, B.A. Rosenzweig, F.D. Sistare. CDER, FDA.

- Dermal Carcinogenicity in Transgenic Mice: Relative Responsiveness of Male and Female Hemizygous and Homozygous Tg.AC Mice to 12-O-Tetradecanoylphorbol 13-Acetate (TPA) and Benzene. K.T. Blanchard, D.J. Ball, H.E. Holden, S.M. Furst, J.H. Stoltz, and R.E. Stoll. Boehringer Ingelheim Pharmaceuticals, Inc.

- Point Mutations of the c-H-ras Gene in Spontaneous Liver Tumors of Transgenic Mice Carrying the Human c-H-ras Gene. S. Hayashi, I. Mori, T. Nonoyama, A. Shino, and K. Mitsumori. Takeda Chemical Industries, Ltd. And National Institute of Health Sciences.

- TGFá is Dispensable for Skin Tumorigenesis. M.C. Humble, C.J. Szczesniak, N.C. Luetteke, J.W. Spalding, R. E. Cannon, L.A. Hansen, D.C. Lee, and R.W. Tennant. University of North Carolina at Chapel Hill, National Cancer Institute and NIEHS.

- Altered Differentiation of Hepatocytes in a Hepatocarcinogenesis Model in Transgenic Mice. A. Enomoto, E.P. Sandgren, and R.R. Maronpot. NIEHS, Institute of Environmental Toxicology and University of Wisconsin.

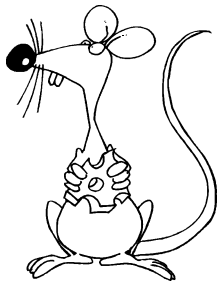
Study Currently Being Conducted at Primedica Redfield Laboratory

By A. Hoberman, L. Lomax and J. Wedig

Primedica Redfield Laboratory is investigating the carcinogenic potential of Cyclosporin A when applied topically, once daily, for 26 weeks to FVB/Tg.AC homozygous transgenic mice as part of the collaborative effort being conducted by HESI's Alternatives to Carcinogenicity Testing Committee. Three groups of 15 male nd 15 female Tg.AC mice are being given cyclosporin A (dissolved in acetone) at 0.05, 0.4 and 0.8 mg/mouse. As a positive control, 15 male and 15 female Tg.AC mice are being given 2.5 ug of TPA topically per day in acetone. In addition, one group of mice of both sexes is receiving 200 ul of acetone topically per day. All of the TPA positive control animals have responded with papillomas at the application site except two females. The percent attrition rate as of June has been:

	Males	Females
Group 1-acetone	20	13
Group 2-TPA	33	20
Group 3-0.05	0	0
Group 4-0.4	33	33
Group 5-0.8	13	13

The reason for the attrition rate across all groups is unknown. The mice treated with cyclosporin A have note shown a positive response dermally when examined grossly. At the end of the 26-week dosing period, the mice will be subjected to a complete histological analysis. Final results are expected to be reported in September 1998.



Contact: J. Wedig at 501-397-2540

Articles of Interest

Goelz, M.F., Mahler, J., Harry, J., Myers, P., Clark, J., Thigpen, J.E., and Forsythe, D.B. (1998) Neuropathologic findings associated with seizures in FVB mice. Lab Animal Science 48(1): 34-37.

Cannon, R.E., Spalding, J.W., Trempus, Szczesniak, C.J., Virgil, K.M., Humble, M.C. and Tennant, R.W. (1998) Kinetics of wound-induced v-Ha-ras transgene expression and papilloma development in transgenic Tg.AC mice. Molecular Carcinogenesis 20: 108-114.

Holden, H., Stoll, R., Spalding, J. and Tennant, R. (1998) The hemizygous Tg.AC transgenic mouse as a potential alternative to the two-year mouse carcinogenicity bioassay: Evaluation of husbandry and housing factors. Journal of Applied Toxicology 18: 19-24.

Study Currently Being Conducted by Purdue Pharma, L.P. and Springborn Laboratories

R.E. Rush, M.S., DABT, Springborn Laboratories, Inc.
J.C. Tigner, Ph.D., Purdue Pharma, L.P.

Springborn Laboratories, Inc. and Purdue Pharma L.P. have engaged in a joint venture to investigate the Tg.AC and p53 transgenic mouse models using the rodent carcinogen methapyrilene hydrochloride. As new members of the ILSI/HESI collaborative research program on alternative models for carcinogenicity testing, we will be examining the effects of dermal exposure in the homozygous Tg.AC mouse strain and or oral (dietary) dosing in the p53+/- mouse strain. Preliminary 1-2 week dose range-finding toxicity/irritation studies have been completed using the wild type strains for Tg.AC and p53 mice (i.e., FVB and C57BL/6, respectively). The FVB mouse was used to examine dermal exposure of methapyrilene in acetone at doses of up to 5000 mg/kg/day (500 mg/ml). No dermal irritation or toxicity was noted at any dose level, although there was difficulty in administering the highest concentrations (300 and 500 mg/ml), due to poor suspendibility at those concentrations. The C57BL/6 mouse was used to evaluate methapyrilene at dietary concentrations of up to 10,000 ppm in PMI rodent meal #5002. Weight loss and decreased food consumption was observed at the highest doses. This will serve as the basis for setting a maximum feasible dose for subsequent range-finding work. Based upon the results of the preliminary studies, we are currently planning the 28-day range-finding studies, which will include toxicokinetics. We plan to initiate these studies in July. We anticipate starting the definitive 26-week studies during the 3rd quarter of 1998 and having results by the 3rd quarter of 1999.

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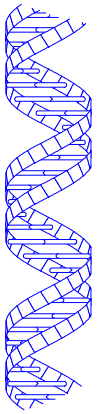
Upcoming Meetings and Events

☺ Validity of Animal Models of Human Respiratory Diseases
Lovelace Respiratory Research Institute, Santa Fe, NM

September 29-October 2, 1998

☺ Gene Environment Interactions: Emerging Issues, Technologies and Biological Paradigms
Barton Creek Conference Resort, Austin, Texas

December 2-5, 1998



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